

APPROVED BY DRAFTSMAN	O.G. FIG.	
	CLASS	SUBCLASS

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Sheet 1 of 23

Title: COLLECTIONS OF BINDING PROTEINS AND TAGS
AND USES THEREOF FOR NESTED SORTING AND
HIGH THROUGHPUT SCREENING.

**Applicant: Ault-Riche et al.
Serial No. 09/910,120 Filed: July 18, 2001
Our Docket No.: 25885-1751**

Sorting by pools

Master

F1

F2

F3

42

42

42

F1₅/F2₃₁/F3₁₆

$$(42)(42)(42) = 74,088$$

FIG. I

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COLLECTIONS OF BINDING PROTEINS AND TAGS
AND USES THEREOF FOR NESTED SORTING AND
HIGH THROUGHPUT SCREENING.
: Ault-Riche et al.

**Applicant: Ault-Riche et al.
Serial No. 09/910,120 Filed: July 18, 2001
Our Docket No.: 25885-1751**

Sorting by pools: Decreasing pool diversities

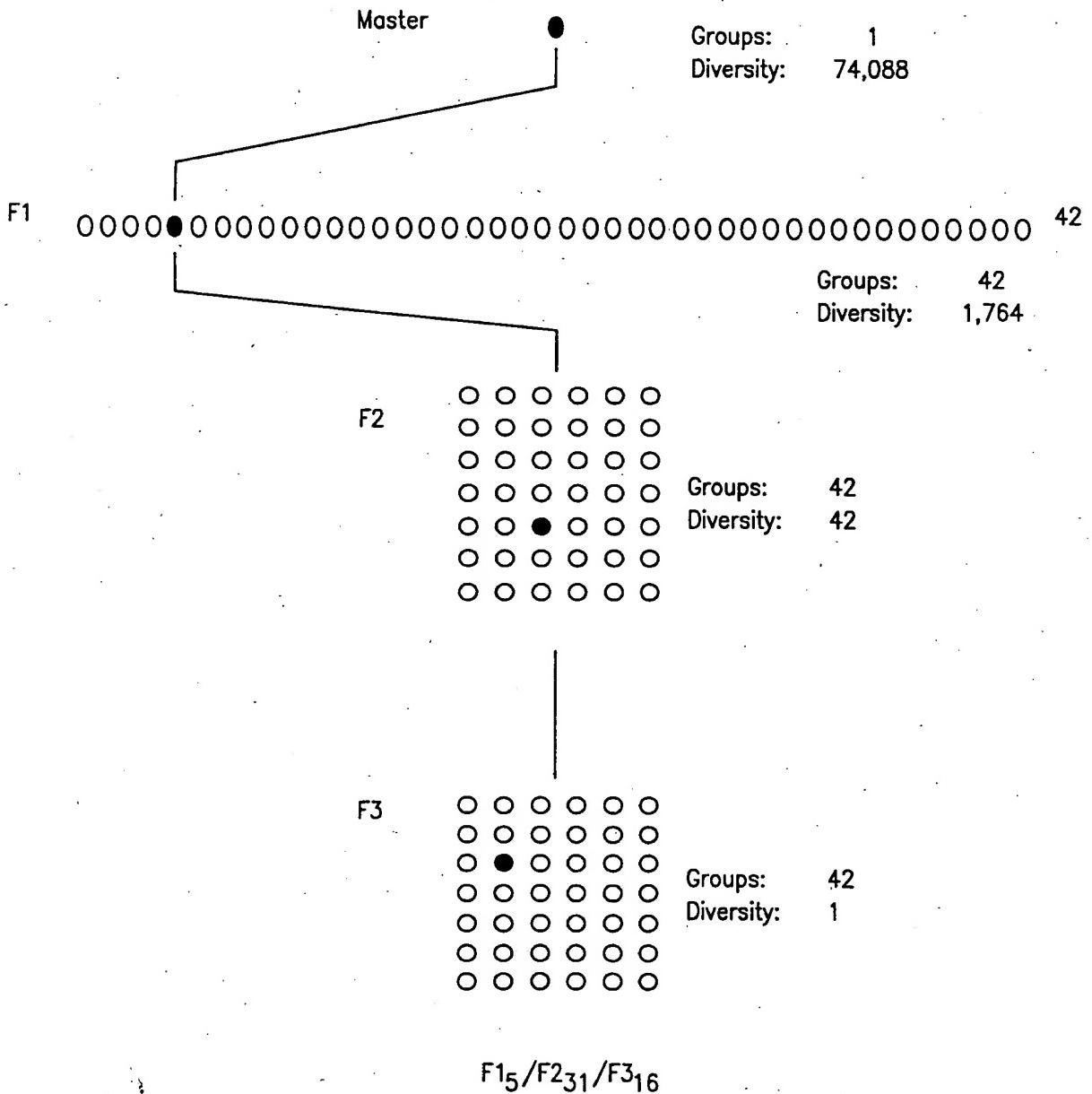


FIG. 2

APPROVED	O.G. FIG.
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Sheet 3 of 23
Title: COLLECTIONS OF BINDING PROTEINS AND THEIR USES THEREOF FOR NESTED SORTING AND HIGH THROUGHPUT SCREENING.
Applicant: Ault-Riche *et al.*
Serial No. 09/910,120 Filed: July 18, 2001
Our Docket No.: 25885-1751

Sorting by pools: Screening large diversity libraries

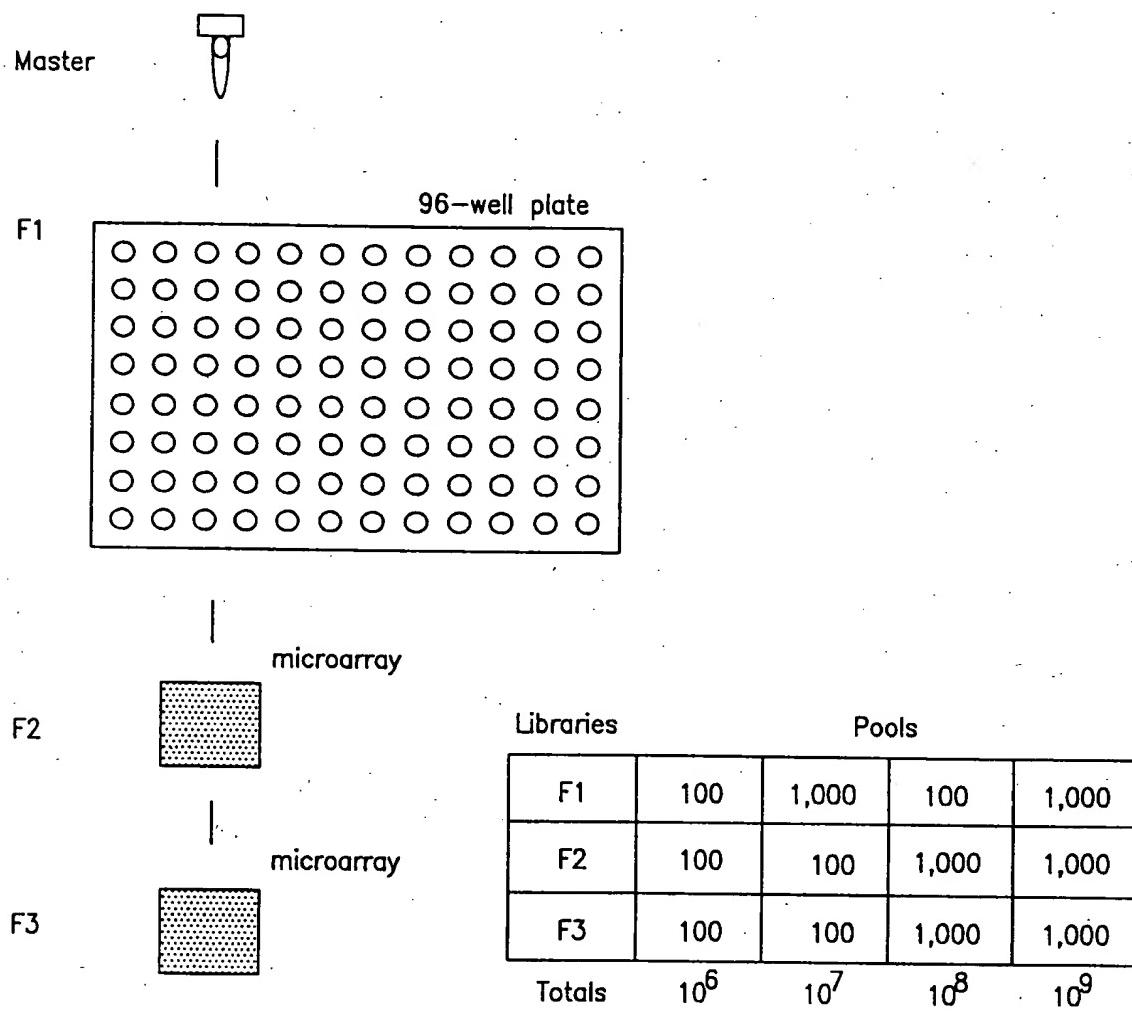


FIG. 3

APPROVED	O.G. FIG.
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COLLECTIONS OF BINDING PROTEINS AND TAGS
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Searching a mutation library

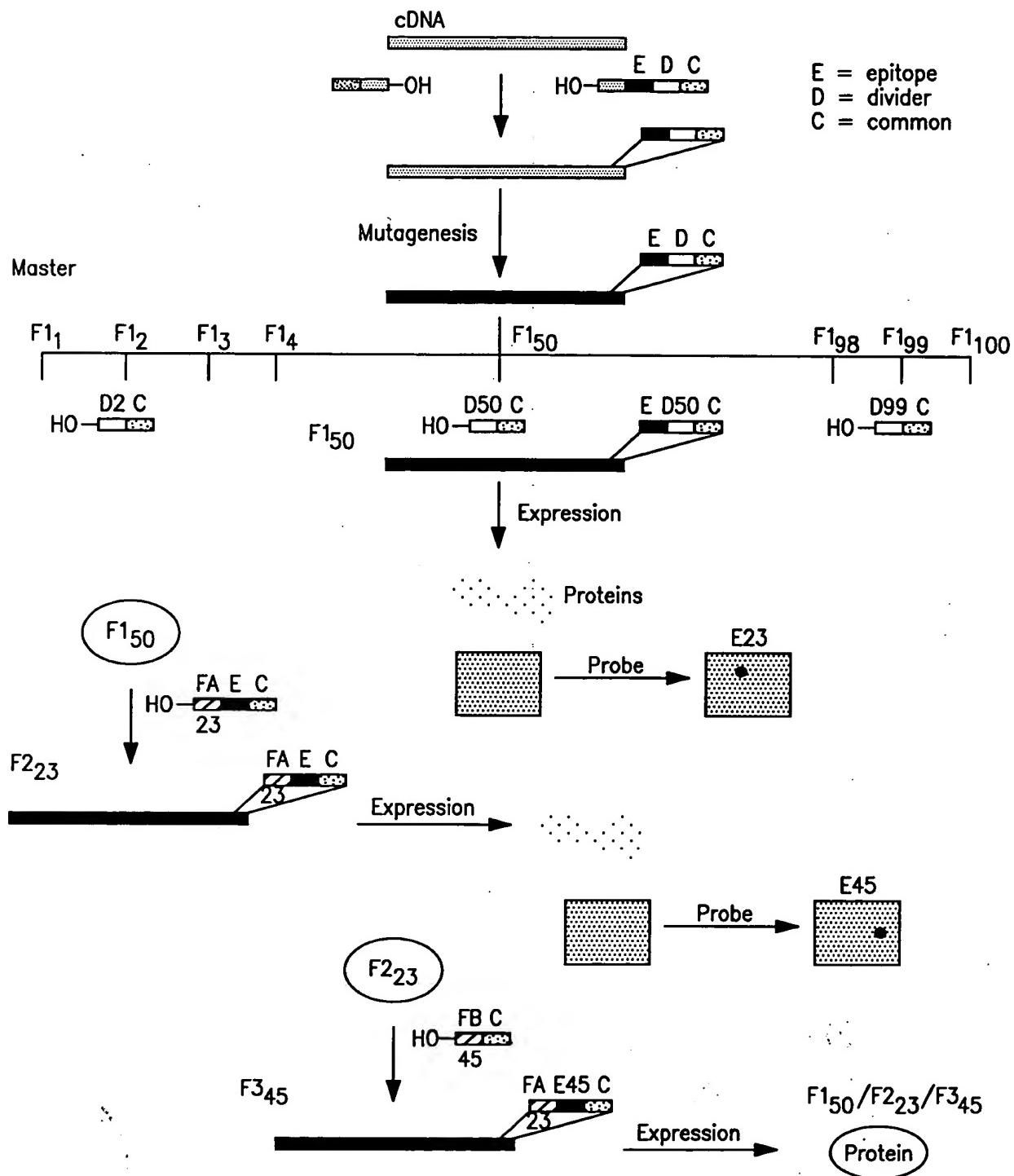
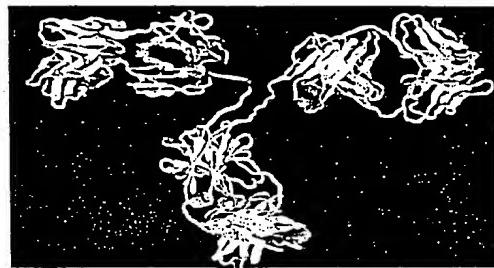
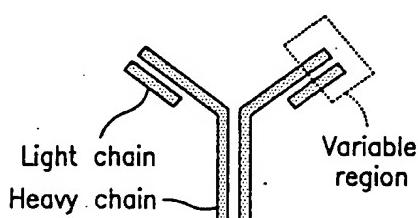


FIG. 4

APPROVED BY DEAFTSMAN	O.G., FIG. CLASS SUBCLASS
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Our Docket No.: 25885-1751

Making a recombinant antibody library



RECOMBINANT ANTIBODY LIBRARY

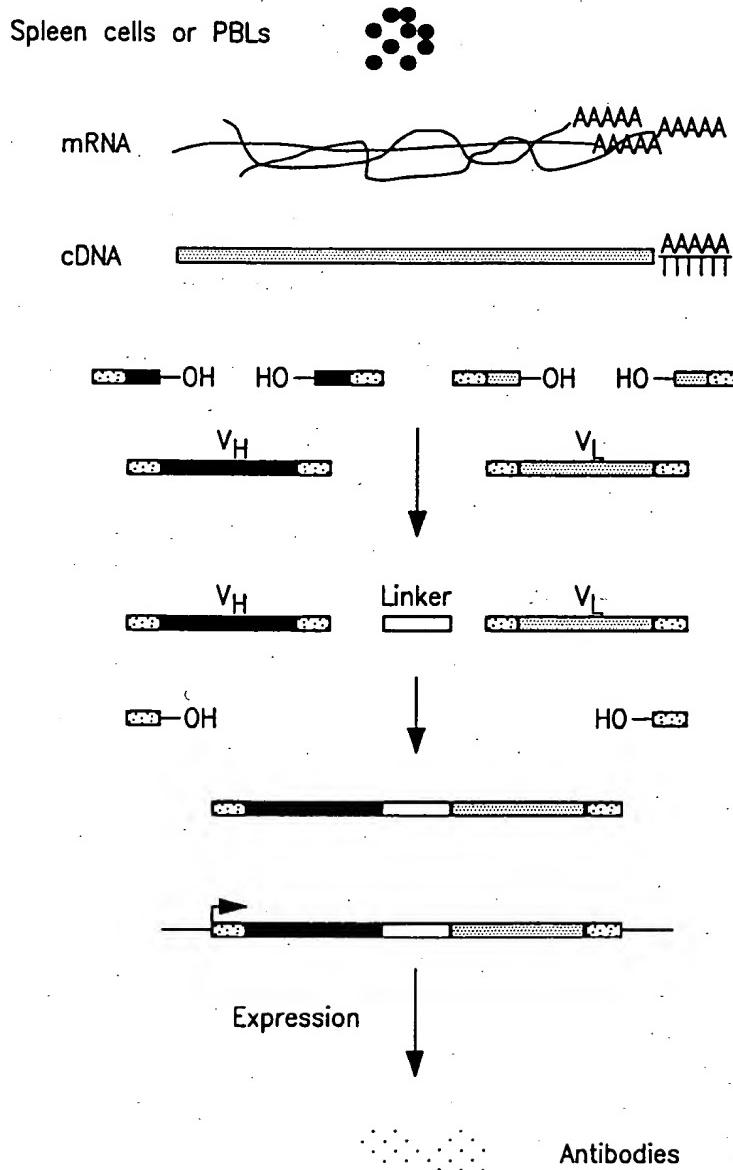


FIG. 5

APPROVED BY DRAFTSMAN	O.G. FIG.
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COLLECTIONS OF BINDING PROTEINS AND TAGS
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Creating the master antibody library: Primer incorporation

1. mRNA purification from spleen or PBLS

..... AAA

V_H



V_L

2. cDNA synthesis

..... AAA

..... AAA

3. amplification

V_{HBACK}

..... AAA

V_{HFOR}

V_{LBACK}

..... AAA

V_{LFOR}

V_{LFOR-C}

4. assembly

V_{HBACK}

V_H

Linker

V_L

E D C

V_{LFOR-C}

V_H Primers

Oligo dT

HO-TTTTTTTT(T)_n
3' 5'

V_{HBACK}

V_H back
OH
5' 3'

V_{HFOR}

J_H for
OH
3' 5'

V_L Primers

V_{LFOR}

J_{kappa} for E D C
HO-
3' 5'

V_{LBACK}

V_{kappa} back
OH
5' 3'

V_{LFOR-C}

C
HO-
3' 5'

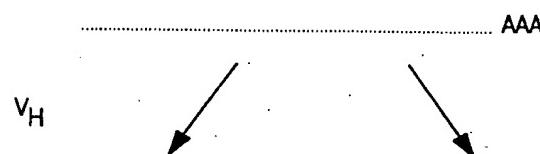
FIG. 6

APPROVED BY DRAFTSMAN	O.G. FIG. CLASS SUBCLASS
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Creating the master antibody library: Linker addition

1. mRNA purification from spleen or PBLs



2. cDNA synthesis



3. amplification



4. assembly

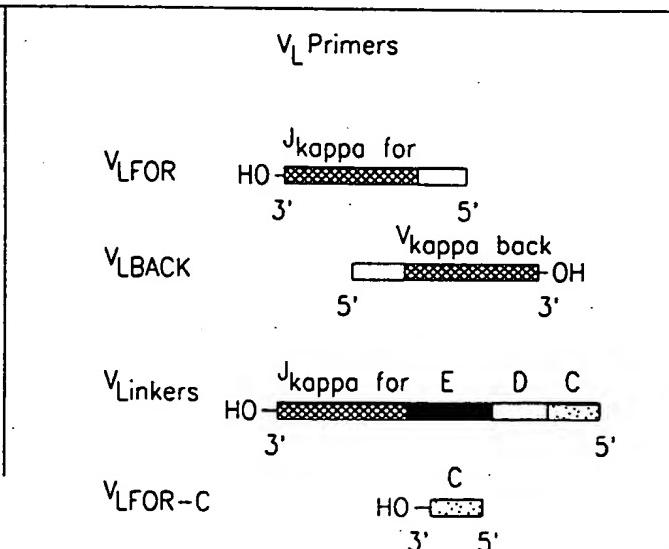
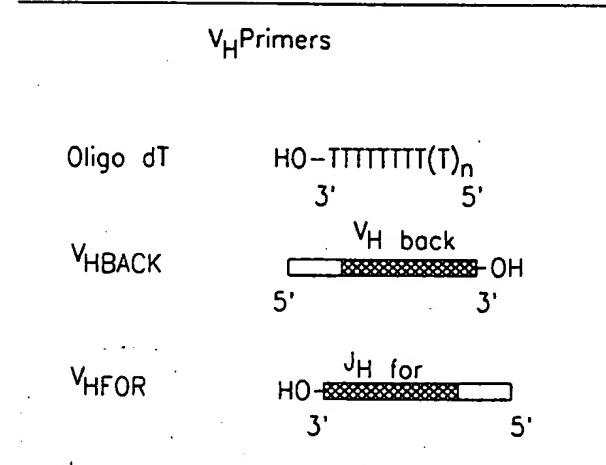
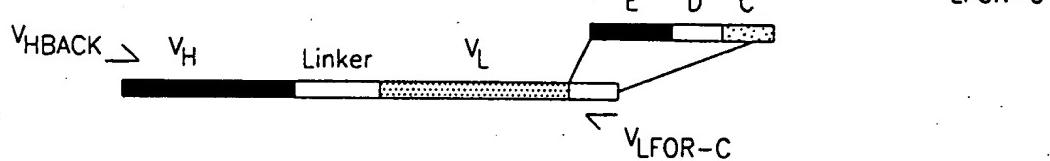


FIG. 7

APPROVED	O.G. FIG.
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 Title: COLLECTIONS OF BINDING PROTEINS AND TAGS
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 Applicant: Ault-Riche et al.
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Searching a recombinant antibody library

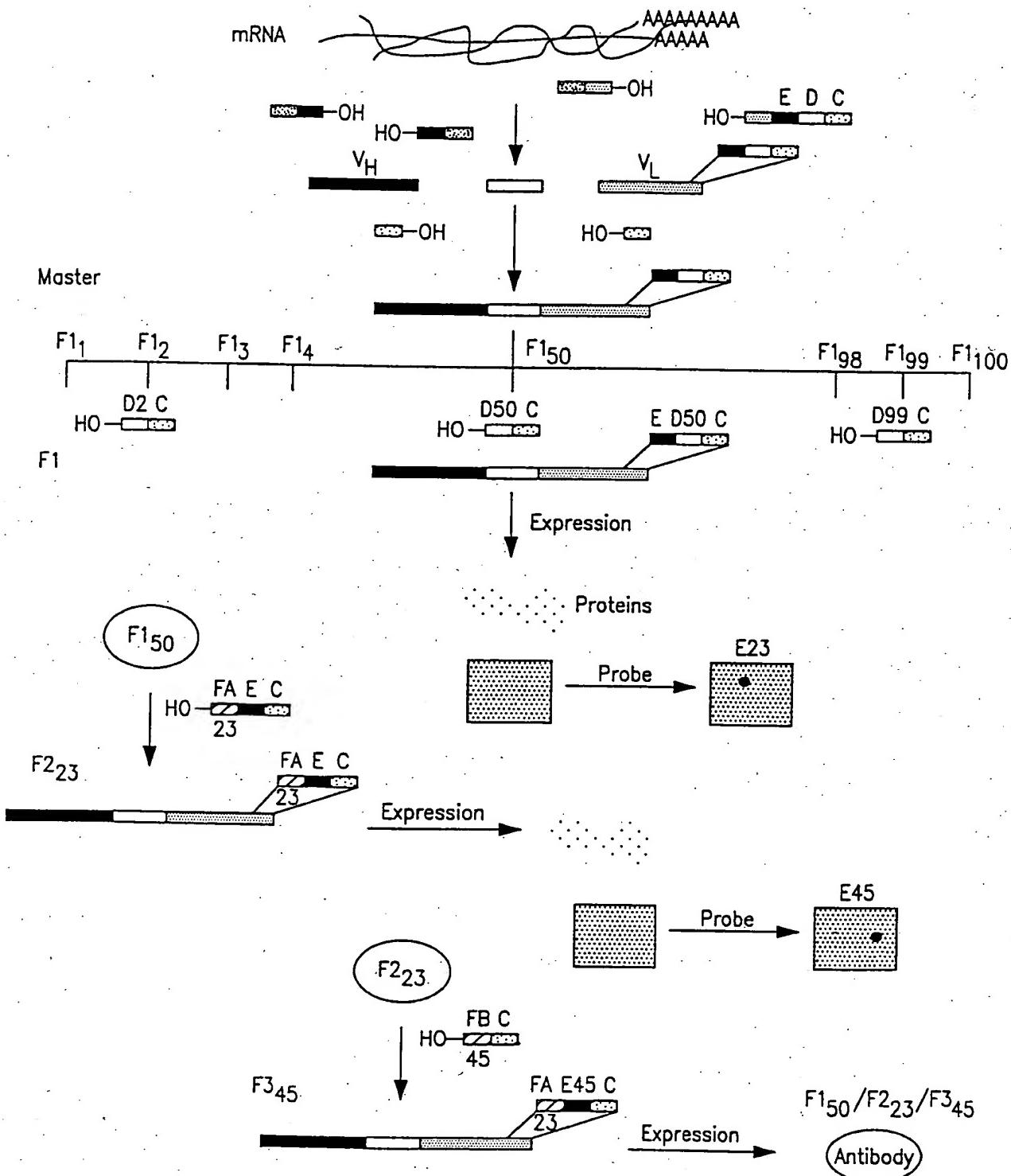


FIG. 8

APPROVED BY DRAFTSMAN	O.G. FIG.
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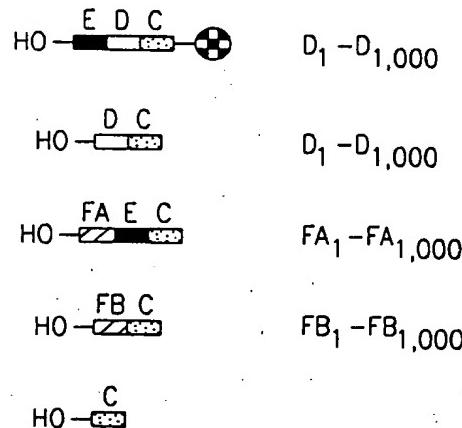
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Applicant: Ault-Riche *et al.*
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Physical elements to include in the kits and combinations

- Anti-tag Arrays™

- Primer sets



- Readers

- Software

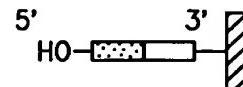
FIG. 9

APPROVED BY DRAFTSMAN	O.G. FIG.	
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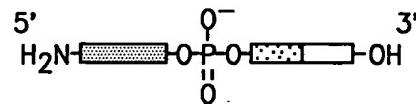
Making the V_{LFOR} primers: Solid phase synthesis



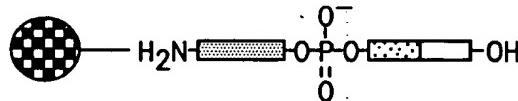
1. Synthesize oligo on solid support



2. Add aminolink prior to cleavage



3. Couple to tosyl activated magnetic beads



4. Extended by hybridizing with DNA patch and ligating

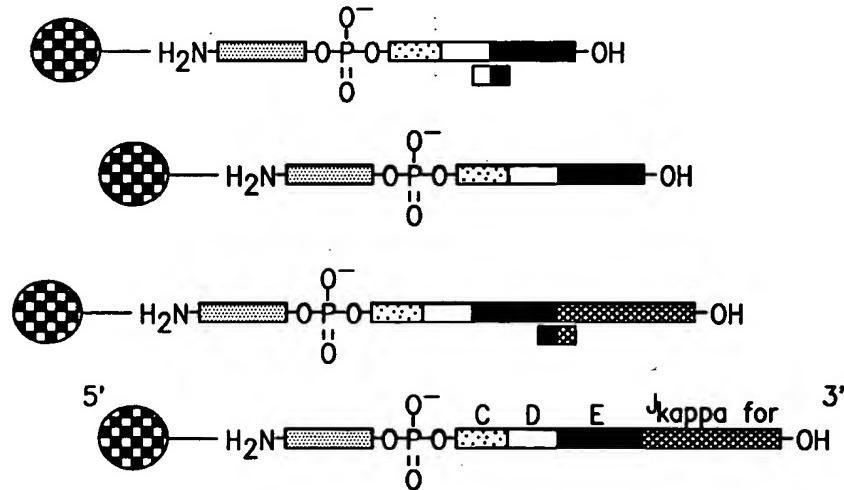


FIG. 10

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Title: COLLECTIONS OF BINDING PROTEINS AND METHODS
AND USES THEREOF FOR NESTED SORTING AND
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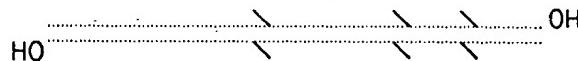
Making the V_{LFOR} primers: Overlapping hybridization



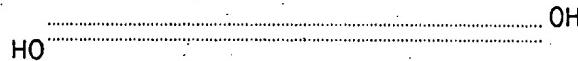
J kappa for Epitope D Common

- Synthesize 4,028 different oligos:
(26 for J kappa for ; 2,000 for Epitope, 2,000 for D; 2 for Common)

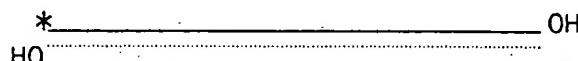
- Assemble oligos for + and - strands of the different regions



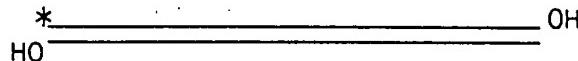
- Ligate the assembled oligos



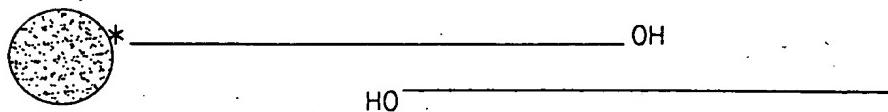
- 1st strand synthesis with biotinylated primer



- 2nd strand synthesis with non-biotinylated primer



- Bind to avidin coated magnetic beads and then denature



- Purify non-biotinylated ssDNA

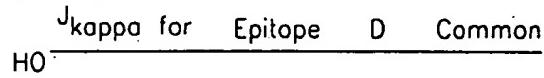


FIG. 11

APPROVED BY DRAFTSMAN	O.G. FIG.
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COLLECTIONS OF BINDING PROTEINS AND TAGS
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Building the collection of antibody/tag pairs: Hybridoma screening

Stable hybridoma cells

Isolated monoclonals
grown in 96-well plates
(quantify Abs in culture
supernatants by ELISA)

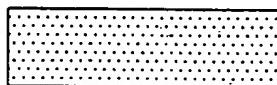
Purified antibodies
(purify with protein L plates;
quantify purified antibodies by ELISA)

Individual protein
preparations

Pooled protein
preparations

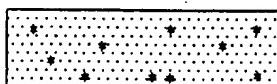
Array onto filter
(up to 10,000 per filter)

Bind purified antibodies to
magnetic beads coated with
anti-mouse Ig mAb (Dynal)



Pan a random disulfide-constrained
phage display library against the beads
(4 rounds with plate amplifications)

Bind enriched phage library to filter;
Stain with anti-phage mAb-HRP;
Image with CCD-based system



Cut out best spots
Recover and propagate phage
(or PCR amplify DNA)

Sequence DNA to
identify epitope tags

FIG. 12

APPROVED BY	O.G. FIG.	
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Table 3 Primers for PCR Amplification of Human Antibody Variable Regions (V genes)

1. V gene primary PCR

A. Human VH back primers (sense)

HuVH1aBACK	5'-CAG GTG CAG CTG GTG CAG TCT GG-3'
HuVH2aBACK	5'-CAC GTC AAC TTA AGG GAG TCT GG-3'
HuVH3aBACK	5'-GAG GTG CAG CTG GTG GAG TCT GG-3'
HuVH4aBACK	5'-CAG GTG CAG CTG CAG GAG TCG GG-3'
HuVH5aBACK	5'-GAG GTG CAG CTG TTG CAG TCT GC-3'
HuVH6aBACK	5'-CAG GTA CAG CTG CAG CAG TCA GG-3'

B. Human JH forward primers (anti-sense)

HuJH1-2FOR	5'-TGA CGA GAC GGT GAC CAG GGT CCC-3'
HuJH3FOR	5'-TGA AGA GAC GGT GAC CAT TGT CCC-3'
HuJH4-5FOR	5'-TGA CGA GAC GGT GAC CAG GGT TCC-3'
HuJH6FOR	5'-TGA CGA GAC GGT GAC CGT GGT CCC-3'

C. Human V kappa back primers (sense)

HuV _k 1aBACK	5'-GAC ATC CAG ATG ACC CAG TCT CC-3'
HuV _k 2aBACK	5'-GAT GTT GTG ATG ACT CAG TCT CC-3'
HuV _k 3aBACK	5'-GAA ATT GTG TTG ACG CAG TCT CC-3'
HuV _k 4aBACK	5'-GAC ATC GTG ATG ACC CAG TCT CC-3'
HuV _k 5aBACK	5'-GAA ACG ACA CTC ACG CAG TCT CC-3'
HuV _k 6aBACK	5'-GAA ATT GTG CTG ACT CAG TCT CC-3'

C. Human V lambda back primers (sense)

HuV _λ 1BACK	5'-CAG TCT GTG TTG ACG CAG CCG CC-3'
HuV _λ 2BACK	5'-CAG TCT GCC CTG ACT CAG CCT GC-3'
HuV _λ 3aBACK	5'-TCC TAT GTG CTG ACT CAG CCA CC-3'
HuV _λ 3bBACK	5'-TCT TCT GAG CTG ACT CAG GAC CC-3'
HuV _λ 4BACK	5'-CAC GTT ATA CTG ACT CAA CCG CC-3'
HuV _λ 5BACK	5'-CAG GCT GTG CTC ACT CAG CCG TC-3'
HuV _λ 6BACK	5'-AAT TTT ATG CTG ACT CAG CCC CA-3'

D. Human J kappa forward primers (anti-sense)

HuJ _k 1FOR	5'-ACG TTT GAT TTC CAC CTT GGT CCC-3'
HuJ _k 2FOR	5'-ACG TTT GAT CTC CAG CTT GGT CCC-3'
HuJ _k 3FOR	5'-ACG TTT GAT ATC CAC TTT GGT CCC-3'
HuJ _k 4FOR	5'-ACG TTT GAT CTC CAC CTT GGT CCC-3'
HuJ _k 5FOR	5'-ACG TTT AAT CTC CAG TCG TGT CCC-3'

D. Human J. lambda forward primers (anti-sense)

HuJ _λ 1FOR	5'-ACC TAG GAC GGT GAC CTT GGT CCC-3'
HuJ _λ 2-3FOR	5'-ACC TAG GAC GGT CAG CTT GGT CCC-3'
HuJ _λ 4-5FOR	5'-ACC TAA AAC GGT GAG CTG GGT CCC-3'

FIG. 13A

APPROVED BY DRAFTSMAN	O.G. FIG.	CLASS	SUBCLASS
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2. Linker fragment PCR

F. Reverse JH for scFv linker (sense)

—FR4 heavy —————>|—linker

RHuJH1-2	5'-GC ACC CTG GTC ACC GTC TCC TCA GGT GG-3'
RHuJH3	5'-GG ACA ATG GTC ACC GTC TCT TCA GGT GG-3'
RHuJH4-5	5'-GA ACC CTG GTC ACC GTC TCC TCA GGT GG-3'
RHuJH6	5'-GG ACC ACG GTC ACC GTC TCC TCA GGT GG-3'

F. Reverse Vk for scFv linker (anti-sense)

—FR1 light —————>|—linker

RHuVklabACKFv	5'-GG AGA CTG GGT CAT CTG GAT GTC CGA TCC GCC-3'
RHuVk2aBACKFv	5'-GG AGA CTG AGT CAT CAC AAC ATC CGA TCC GCC-3'
RHuVk3aBACKFv	5'-GG AGA CTG CGT CAA CAC AAT TTC CGA TCC GCC-3'
RHuVk4aBACKFv	5'-GG AGA CTG GGT CAT CAC GAT GTC CGA TCC GCC-3'
RHuVk5aBACKFv	5'-GG AGA CTG CGT GAG TGT CCT TTC CGA TCC GCC-3'
RHuVk6aBACKFv	5'-GG AGA CTG AGT CAG CAC AAT TTC CGA TCC GCC-3'

F. Reverse V λ for scFv linker (anti-sense)

—FR1 light —————>|—linker

RHuV\lBACK1Fv	5'-GG CGG CTG CGT CAA CAC AGA CTG CGA TCC GCC ACC GCC AGA G-3'
RHuV\lBACK2Fv	5'-GC AGG CTG AGT CAG AGC AGA CTG CGA TCC GCC ACC GCC AGA G-3'
RHuV\lBACK3aFv	5'-GG TGG CTG AGT CAG CAC ATA GGA CGA TCC GCC ACC GCC AGA G-3'
RHuV\lBACK3bFv	5'-GG CTG CTG AGT CAG CTC AGA AGA CGA TCC GCC ACC GCC AGA G-3'
RHuV\lBACK4Fv	5'-GG CGG TTG AGT CAG TAT AAC GTG CGA TCC GCC ACC GCC AGA G-3'
RHuV\lBACK5Fv	5'-GA CGG CTG AGT CAG CAC AGA CTG CGA TCC GCC ACC GCC AGA G-3'
RHuV\lBACK6Fv	5'-TG GGG CTG AGT CAG CAT AAA ATT CGA TCC GCC ACC GCC AGA G-3'

3. Pull-through primers for introduction of restriction sites*

G. Human VH back (Sfi)primers (sense)

|—FR1 heavy —————

HuVH1aBACKSf1	5'-GTC CTC GCA ACT <u>GGG</u> <u>CCC</u> CAG <u>CGG</u> <u>GCC</u> ATG GCC CAG GTG CAG CTG GTG CAG TCT GG-3'
HuVH2aBACKSf1	5'-GTC CTC GCA ACT <u>GGG</u> <u>CCC</u> CAG <u>CGG</u> <u>GCC</u> ATG GCC CAG GTC AAC TTA AGG GAG TCT GG-3'
HuVH3aBACKSf1	5'-GTC CTC GCA ACT <u>GGG</u> <u>CCC</u> CAG <u>CGG</u> <u>GCC</u> ATG GCC GAG GTG CAG CTG GTG GAG TCT GG-3'
HuVH4aBACKSf1	5'-GTC CTC GCA ACT <u>GGG</u> <u>CCC</u> CAG <u>CGG</u> <u>GCC</u> ATG GCC CAG GTG CAG CTG CAG GAG TCG GG-3'
HuVH5aBACKSf1	5'-GTC CTC GCA ACT <u>GGG</u> <u>CCC</u> CAG <u>CGG</u> <u>GCC</u> ATG GCC CAG GTG CAG CTG TTG CAG TCT CC-3'
HuVH6aBACKSf1	5'-GTC CTC GCA ACT <u>GGG</u> <u>CCC</u> CAG <u>CGG</u> <u>GCC</u> ATG GCC CAG GTA CAG CTG CAG CAG TCA GG-3'

H. Human J kappa forward (Not) primers (anti-sense)

|—FR4 light —————

HuJk1FORNot	5'-GAG TCA TTC TCG ACT <u>TGC</u> <u>GGC</u> <u>CCC</u> ACG TTT GAT TTC CAC CTT GGT CCC-3'
HuJk2FORNot	5'-GAG TCA TTC TCG ACT <u>TGC</u> <u>GGC</u> <u>CCC</u> ACG TTT GAT CTC CAG CTT GGT CCC-3'

H. Human J kappa forward (Not) primers (anti-sense)(continued)

|—FR4 light —————

HuJk3FORNot	5'-GAG TCA TTC TCG ACT <u>TGC</u> <u>GGC</u> <u>CCC</u> ACG TTT GAT ATC CAC TTT GGT CCC-3'
HuJk4FORNot	5'-GAG TCA TTC TCG ACT <u>TGC</u> <u>GGC</u> <u>CCC</u> ACG TTT GAT CTC CAC CTT GGT CCC-3'
HuJk5FORNot	5'-GAG TCA TTC TCG ACT <u>TGC</u> <u>GGC</u> <u>CCC</u> ACG TTT AAT CTC CAG TCG TGT CCC-3'

H. Human J lambda forward (Not) primers (anti-sense)

FR4 light

HuJ11FORNOT	5'-GAG TCA TTC TCG ACT <u>TGC</u> <u>GGC</u> <u>CCC</u> ACC TAG GAC GGT GAC CTT GGT CCC-3'
HuJ12-3FORNOT	5'-GAG TCA TTC TCG ACT <u>TGC</u> <u>GGC</u> <u>CCC</u> ACC TAG GAC GGT CAG CTT GGT CCC-3'
HuJ14-5FORNOT	5'-GAG TCA TTC TCG ACT <u>TGC</u> <u>GGC</u> <u>CCC</u> ACC TAA AAC GGT GAG CTG GGT CCC-3'

*Recognition site for restriction enzyme is underlined.

FIG. 13B

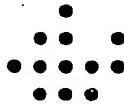
APPROVED	O.G. FIG.	
BY	CLASS	SUBCLASS
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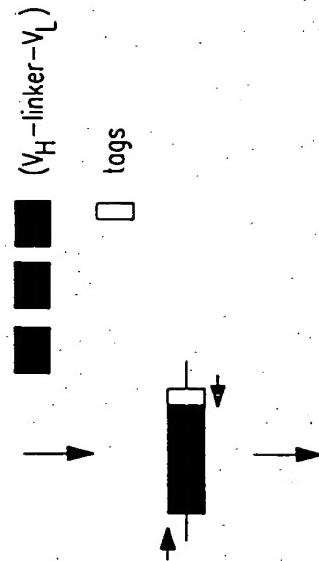
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step I

Tag and assemble immunoglobulin genes



Create 1,000 sub-libraries by separate PCR amplification
reactions using tag-specific PCR primers

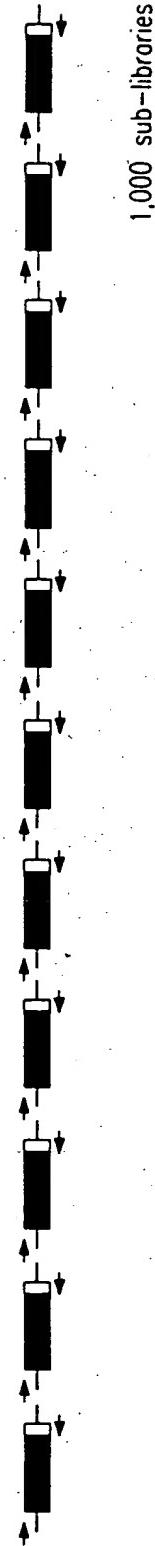


FIG. 14A

APPROVED BY DRAFTSMAN	O.G. FIG.
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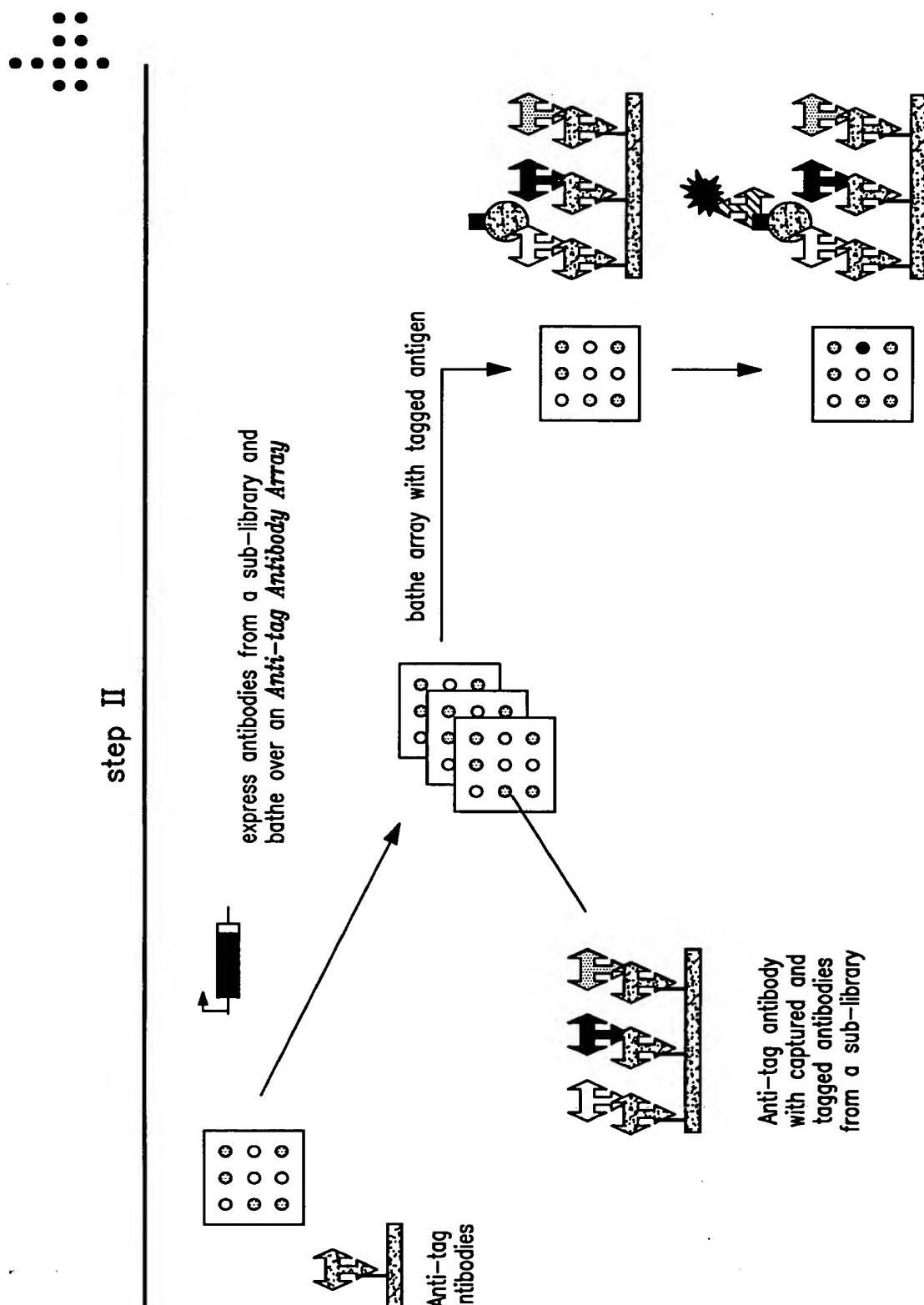


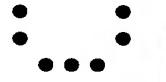
FIG. 14B

APPROVED	O.G. FIG.	
BY	CLASS	SUBCLASS
DRAFTSMAN		

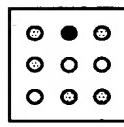
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Title: COLLECTIONS OF BINDING PROTEINS AND TAGS
AND USES THEREOF FOR NESTED SORTING AND
HIGH THROUGHPUT SCREENING.

Applicant: Ault-Riche *et al.*
Serial No. 09/910,120 Filed: July 18, 2001
Our Docket No.: 25885-1751



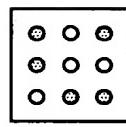
step III



Amplify the antibody genes from the identified sub-library using tag-specific PCR primers

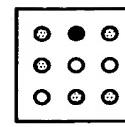
If the starting diversity of the master library was 1,000,000,000 then each spot in this array will have 1,000 different types of rAbs

Express and purify the antibodies



Re-distribute over an Anti-tag Antibody Array

If the starting diversity of the master library was 1,000,000,000 then each spot in this array will have a single type of rAb



Re-survey to ID the antibody of interest

FIG. 14C

APPROVED BY DRAFTSMAN	O.G. FIG.	
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summary

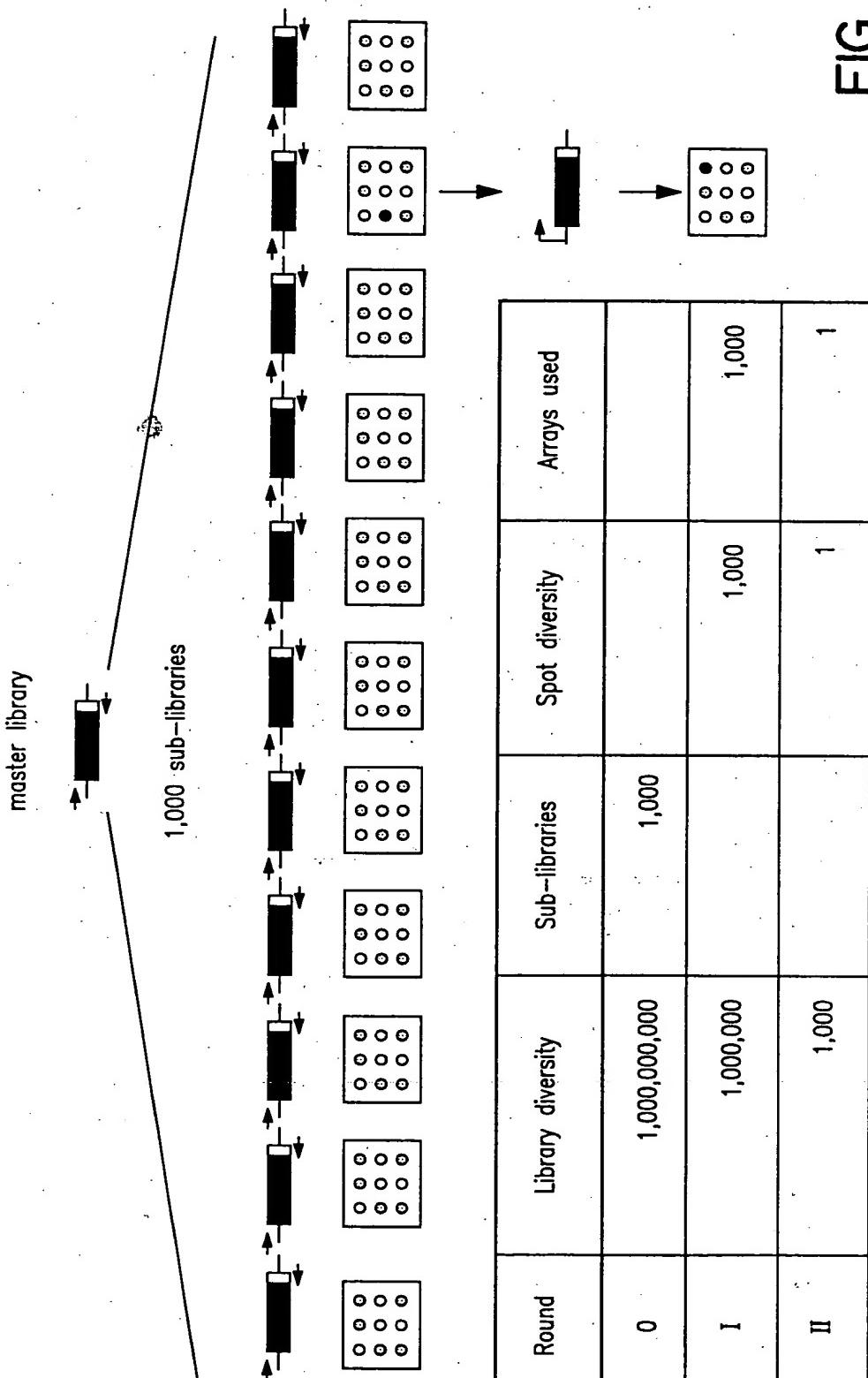


FIG. 14D

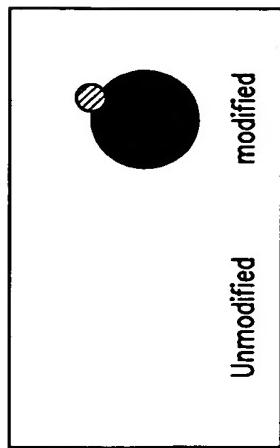
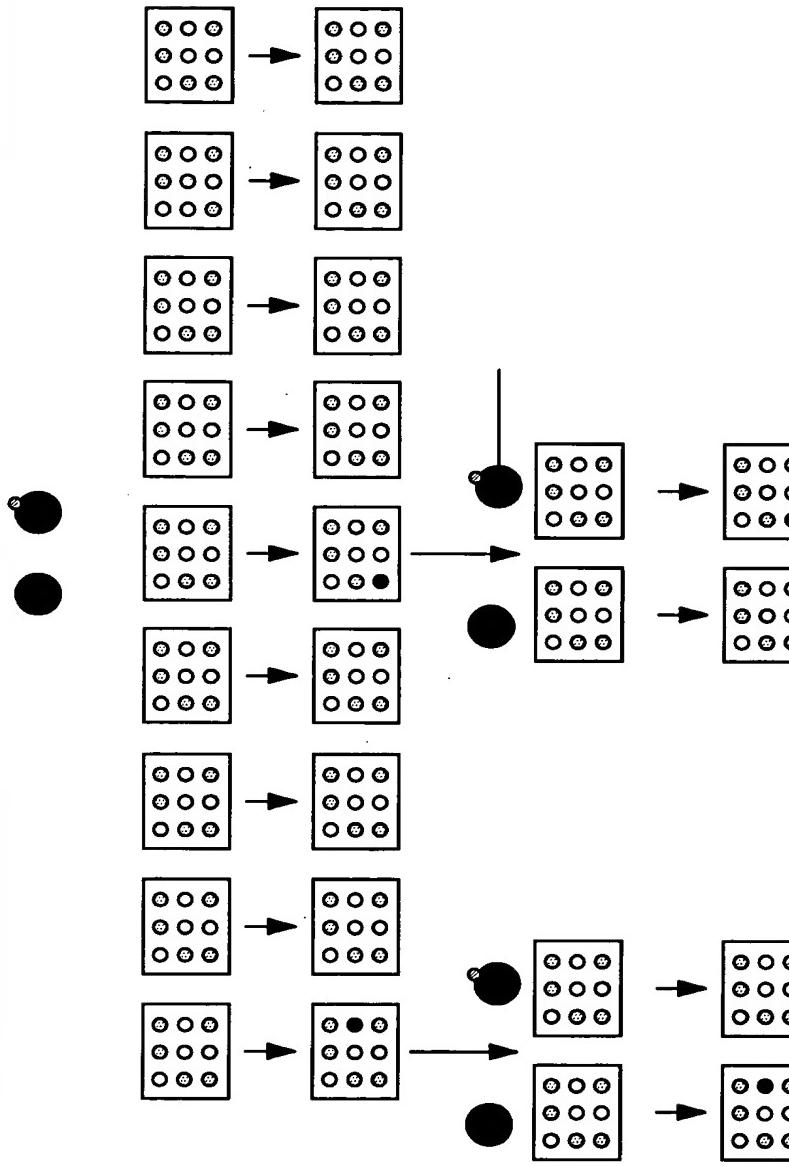
APPROVED BY DRAFTSMAN	O.G. FIG.
	CLASS SUBCLASS

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FIG. 15

Modification searches



APPROVED BY	O.G. FIG.	
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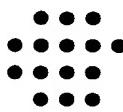
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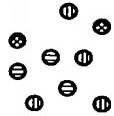
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FIG. 16

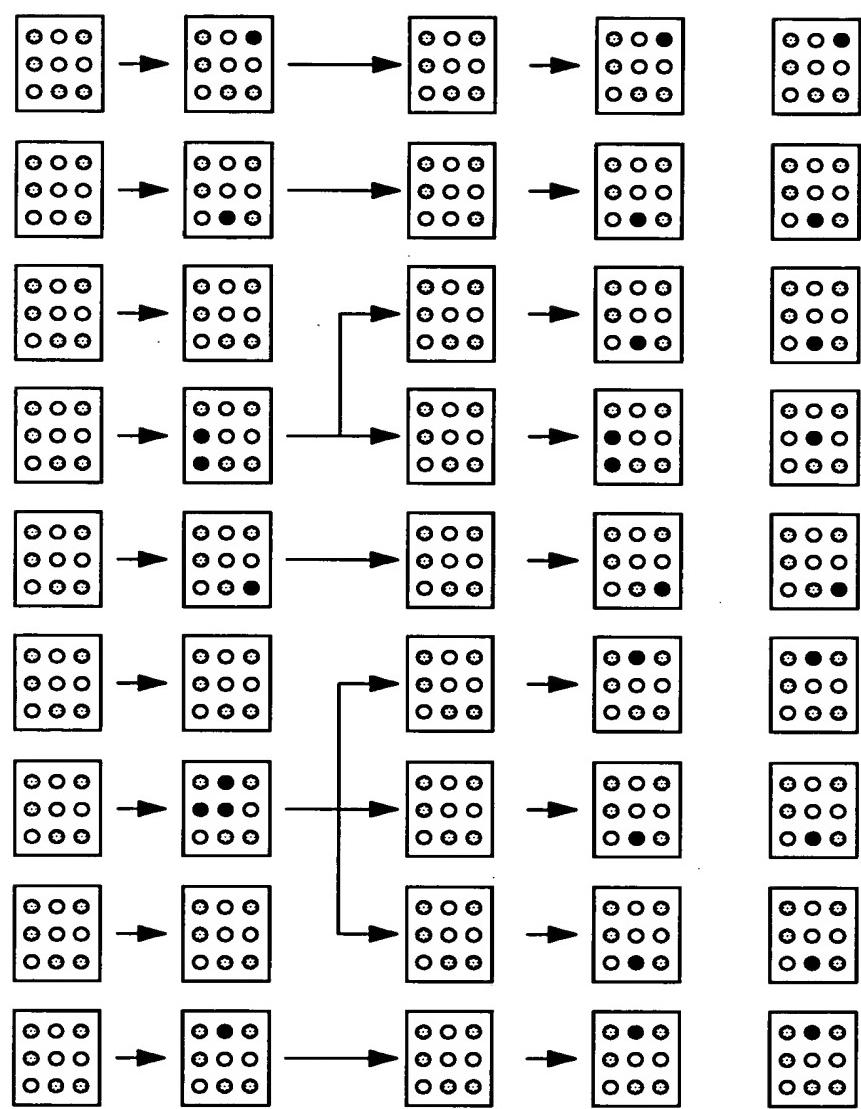


Simultaneous searches



Round Arrays Bait Probe

I 1,000 Abs Ags



3 Arrays per Ag

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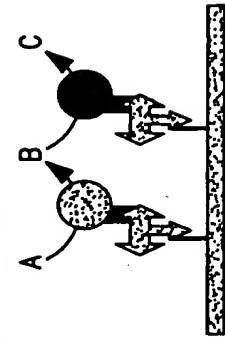
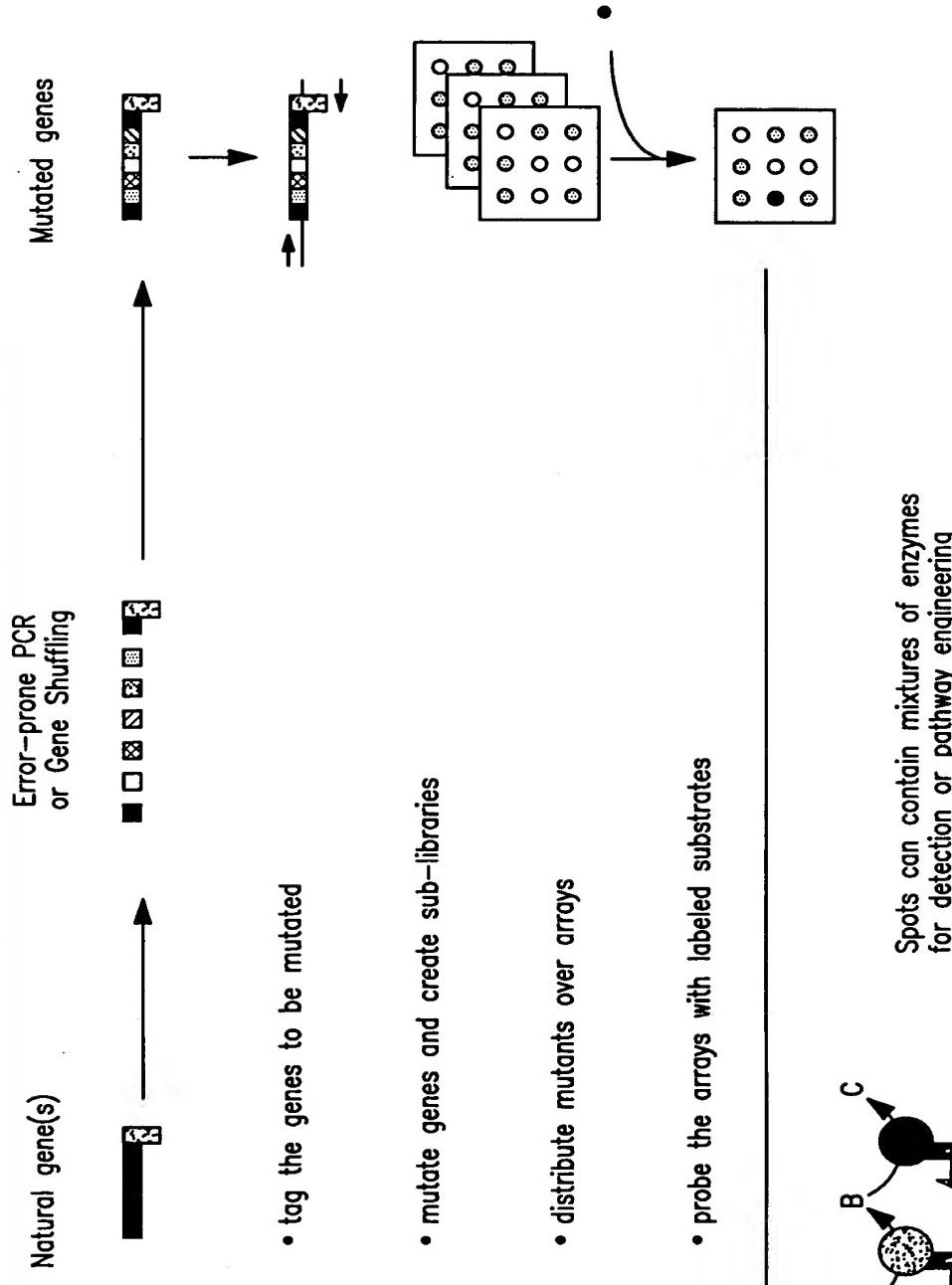
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Protein interaction mapping



Spots can contain mixtures of enzymes for detection or pathway engineering

17

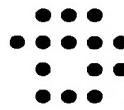
APPROVED BY	O.G. FIG.	
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Protein interaction mapping

cDNA library

- Human tissue
 - pathogen
 - yeast
- Generate a tagged cDNA library
-

Create sub-libraries by PCR

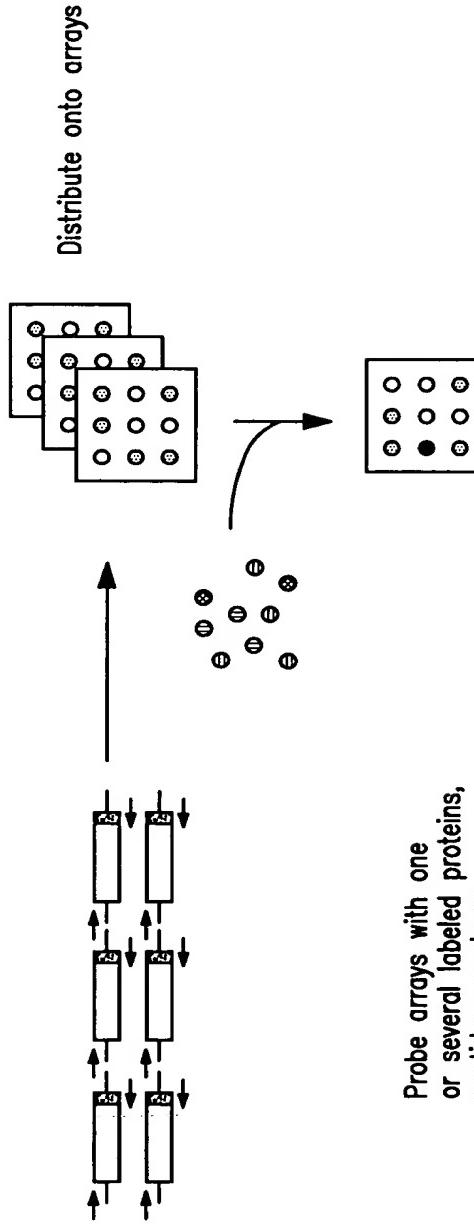


FIG. 18

APPROVED BY	O.G. FIG.	
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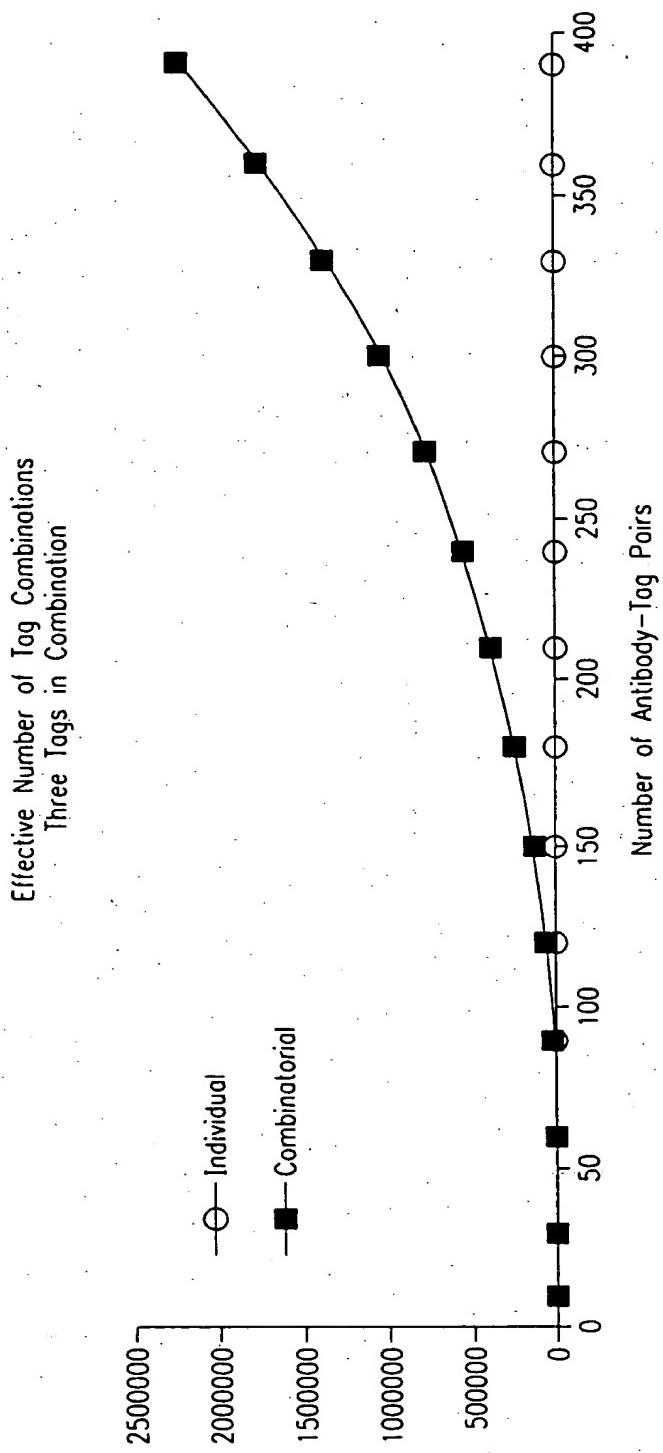


FIG. 19